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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 2 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **DIFETHIALONE**: Review of a developmental (teratology) study in the rabbit. **NEW CHEMICAL**
EPA DP Barcode D217613; EPA Submission No. S490748; MRID No: 43720301; EPA Pesticide Chemical Code 128967; Toxicology Chemical No. 114AAB.

TO: Robert Forrest/Daniel Peacock, PM 14
Insecticide-Rodenticide Branch/RD (7505C)
and
Debbie McCall
RCAB/HED (7509C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 4/23/96
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y. M Ioannou 4/23/96
Section Head, Review Section I
and
Stephanie R. Irene, Ph.D.
Acting Chief, Toxicology Branch II
Health Effects Division (7509C) Stephanie R. Irene 4/26/96

Registrant: LiphaTech, Inc.
3600 West Elm Street, Milwaukee, WI 53209

Action Requested: Review a developmental (teratology) study in the rabbit with Difethialone.

Recommendations: TBII has reviewed the study *Developmental Toxicity Evaluation of Difethialone Administered by Gavage to New Zealand White Rabbits* (Reproductive and Developmental Toxicity Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute for LiphaTech, Inc., RTI Identification No.: 65C-5724-03/04, October 26, 1994, EPA MRID# 43720301), the following is the executive summary from the review of the study:

In a developmental toxicity (teratology) study (MRID# 43720301), New-Zealand White Rabbits from Hazleton Research Products, Inc., Denver, PA received either 0, 2.5, 5.0 10.0, or 20.0 µg/kg/day

Difethialone Technical (Lot No. DIF8011; Purity - 100.95%) in corn oil by oral gavage from gestation days 7 through 19, inclusive.

Maternal toxicity was noted in the high mid and high dose groups as increased mortality with associated clinical observations related to hemorrhage prior to death. It is noted that maternal toxicity was noted at 15.0 µg/kg/day and above in the range-finding study. **The Maternal Toxicity NOEL is 5.0 µg/kg/day and the Maternal Toxicity LOEL = 10.0 µg/kg/day based on increased mortality and clinical signs of toxicity.**

Since all dams died in the high dose, possible developmental toxicity could not be determined for that dose; therefore, this will be considered as the developmental toxicity LOEL. It is noted that in the range-finding study at 15.0 µg/kg/day and above there was an increase in resorptions/litter, nonlive/litter and nonlive plus malformed/litter; however, only 5 dams per dose group were used. **The Developmental Toxicity NOEL = 10.0 µg/kg/day and the Developmental Toxicity LOEL = 20.0 µg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§83-3b) for a developmental toxicity (teratology) study in rabbits.

I. Toxicology Profile for Difethialone

Technical: Difethialone
Action Type: New chemical

This compound is an unregistered active ingredient. The following data are required for Difethialone Technical.

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	Yes
§81-4 Primary eye irritation in rabbits	Yes	Yes
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization in guinea pigs	Yes	Yes
§81-7 Acute Neurotoxicity - hen	No	
§81-8 Acute Neurotoxicity - mammal	No	
§82-1(a) Subchronic Oral (rodent)	Yes	Yes
§82-1(b) Subchronic Oral (nonrodent)	No	
§82-5 Subchronic Neurotoxicity	No	
§82-2 21 Day Dermal - rat	No	
§83-1(a) Chronic toxicity (rodent)	No	
§83-1(b) Chronic Toxicity (nonrodent)	No	
§83-2(a) Carcinogenicity - rat	No	
§83-2(b) Carcinogenicity - mouse	No	
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	No	Yes[1]
§83-4 Multigeneration reproduction-rat	No	
§84-2(a) Mutagenicity-Gene Mutation	Yes	Yes
§84-2(b) Mutagenicity-Struct. Chrom. Aberr.	Yes	Yes
§84-4 Mutagenicity-Other Genotox. Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes[2]
§85-2 Dermal Penetration		
§86-1 Domestic Animal Safety	Yes	Yes

[1] = reviewed in this memo

[2] = modified metabolism study

II. Data Gaps

The are no data gaps for Difethialone Technical at this time.

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. Reference Dose

No RfD has been established for this chemical.

V. Pending Regulatory Actions

None at this time.

VI. Toxicological Issues Pertinent to this Request

This chemical is a new chemical.

A. New toxicology Data on Difethialone Technical

Data reviews are discussed in this action.

B. Carcinogenicity and mutagenicity

No data are available for this compound for carcinogenicity. Difethialone Technical was negative in two chromosome aberration assays in human lymphocytes, in a mouse micronucleus assay , a mammalian microsome plate incorporation assay, and in a HGPRT assay in CHO cells.

DIFETHIALONE PRENATAL DEVELOPMENTAL TOXICITY (TERATOLOGY)-RABBIT
OPPTS 870.3700; OPP §83-3A

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 4/17/96
Senior Pharmacologist, Review Section I, TBII (7509C)

Secondary Review by: James N. Rowe, Ph.D. James N. Rowe 4/17/96
Section Head, Review Section III, TBII (7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Prenatal Developmental Toxicity Study
Species: Rabbit; Guideline: OPPTS 870.3700, OPP §83-3a

EPA Numbers: EPA MRID# 43720301
EPA Pesticide Chemical Code 128967
Toxicology Chemical No. 114AAB
EPA DP Barcode D217613
EPA Submission Barcode S490748
Reregistration Case# NONE

Test Material: Difethialone Technical
Synonyms: [(Bromo-4'-[Biphenyl-1-1']-yl-4)3-Tetrahydro-1,2,3,4-Naphthyl-1]3-Hydroxy-4,2H-1-Benzothiopyran-one-2; CAS No. 104653-34-1

Title of Report: Developmental Toxicity Evaluation of Difethialone Administered by Gavage to New Zealand White Rabbits

Sponsor: LiphaTech, Inc., 3600 W. Elm Street, Milwaukee, WI 53209

Testing Facility: Reproductive and Developmental Toxicity Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709-2194

Study Number: RTI Identification No.: 65C-5724-03/04

Author(s): Rochelle W. Tyl, Ph.D. D.A.B.T., Melissa C. Marr, B.A., L.A.T.G., Christina B. Myers, M.S.

Report Issued: October 26, 1994

Executive Summary: In a developmental toxicity (teratology) study (MRID# 43720301), New-Zealand White Rabbits from Hazleton Research Products, Inc., Denver, PA received either 0, 2.5, 5.0 10.0, or 20.0 µg/kg/day Difethialone Technical (Lot No. DIF8011; Purity - 100.95%) in corn oil by oral gavage from gestation days 7 through 19, inclusive.

Maternal toxicity was noted in the high mid and high dose groups

as increased mortality with associated clinical observations related to hemorrhage prior to death. It is noted that maternal toxicity was noted at 15.0 µg/kg/day and above in the range-finding study. **The Maternal Toxicity NOEL is 5.0 µg/kg/day and the Maternal Toxicity LOEL = 10.0 µg/kg/day based on increased mortality and clinical signs of toxicity.**

Since all dams died in the high dose, possible developmental toxicity could not be determined for that dose; therefore, this will be considered as the developmental toxicity LOEL. It is noted that in the range-finding study at 15.0 µg/kg/day and above there was an increase in resorptions/litter, nonlive/litter and nonlive plus malformed/litter; however, only 5 dams per dose group were used. **The Developmental Toxicity NOEL = 10.0 µg/kg/day and the Developmental Toxicity LOEL = 20.0 µg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§83-3b) for a developmental toxicity (teratology) study in rabbits.

Compliance

A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, GLP Compliance Statement, FIFRA FLAGGING STATEMENT (the study neither meets nor exceeds any of the applicable criteria), and a QUALITY ASSURANCE STATEMENT was provided.

A. Materials and Methods: A copy of the Materials and Methods section from the investigators report is attached.

1. Test compound: Difethialone Technical
Description - white-pinkish odorless powder
Lot No. DIF8011
Purity - 100.95% a.i.
CAS No. 104653-34-1
Received - 9/13 and 10/21/93 and 3/17/94
Contaminants - none reported

2. Vehicle(s): Corn oil (Mazola®, CAS No. 8001-30-7)

3. Test animals: Species: Rabbit
Strain: New-Zealand white
Age: 5 months on gd 0
Weight: 2792-4158 g on gd 0
Source: Hazleton Research Products, Inc., Denver, PA
ANIMALS WERE TIMED PREGNANT (NATURALLY MATED)

B. Study Design

This study was designed to assess the developmental toxicity potential of Difethialone Technical when administered by oral gavage to pregnant New-Zealand White Rabbits on gestation days 7 through 19, inclusive.

1. Mating Procedure

Animals were timed pregnant, naturally mated at Hazleton Research Products, Inc., Denver, PA.

2. Animal Husbandry

Animals were kept under standard animal care conditions and since they were timed pregnant, they were not acclimated to the laboratory conditions before mating; however they had 5-6 days prior to dosing initiation to acclimatize. They received #5322 Purina Certified Rabbit Chow®, initially rationed then *ad libitum* from gestation day 3 on and water (deionized/filtered tap water in bottles) *ad libitum*.

3. Group Arrangement

The animals were randomly allocated ...*by a stratified randomization method to provide uniform mean body weights across dose groups on gestation day 3*...to the following groups:

Test Group	Dose Level (µg/kg/day)	Number Assigned
Control	0	16
Low Dose	2.5	16
Low Mid Dose	5.0	16
High Mid Dose	10.0	16
High Dose	20.0	16

Doses were based on a range finding study with the following results (scanned from pages 9 and 10 of the investigators report):

A range-finding study was performed to establish the dose-response range of difethialone in New Zealand White rabbits for use in the developmental toxicity study. A detailed report of this rangefinding study can be found in Appendix V. The study design involved five (5) treatment groups and a vehicle control group, each comprised of five (5) rabbits at doses of 0.0, 1.25, 2.5, 5.0, 15.0 and 30.0 µg/kg/day. In addition, there were four (4) treatment groups and a vehicle control group, each comprised of three (3) naturally bred rabbits designated as satellite females, at doses of 0.0, 0.625, 1.25, 2.5 and 5.0 µg/kg/day. The dosing period was gd 7 through 19. Satellite females were handled, treated and examined exactly like study females until gd 20 at which time satellite females were euthanized for Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) analyses. On gd 30, all surviving study females were euthanized, weighed and necropsied. All live fetuses were weighed, examined for external malformations (including cleft palate) and variations, and discarded.

Maternal mortality and morbidity were observed at 15.0 and 30.0 µg/kg/day. There were no treatment- or dose-related clinical observations at doses of 0.0 through 5.0 µg/kg/day. The clinical signs seen at 15.0 and 30.0 µg/kg/day were internal bleeding externalized from the nose and/or mouth and vagina, external bleeding at the site of the eartag, pallor and ultimately morbidity and death. There were no changes in PT or APTT in any dose examined. At 15.0 µg/kg/day, there were significant increases in resorptions per litter, in nonlive (resorptions plus dead) per litter and in adversely affected (nonlive plus malformed) per litter, all due to the increased resorptions. The percentage of litters with one or more resorptions exhibited a dose related upward trend and the percentage of litters with nonlive and adversely affected implants was significantly increased at 15.0 µg/kg/day. There were no treatment- or dose-related changes in pre- or post-implantation loss, incidence of nonlive or adversely affected implants per litter, of the number of live fetuses per litter, of fetal body weight per litter in any of the dose groups, 0.0 through 5.0 µg/kg/day. Litter size and mean fetal body weight per

litter were reduced at 15.0 µg/kg/day. External examination of all live fetuses indicated no malformations or variations detected in this study.

In this preliminary study, doses of 30.0 or 15.0 µg/kg/day were toxic to the does, resulting in high levels of mortality. In contrast, doses of 1.25 through 5.0 µg/kg/day resulted in no detectable maternal or developmental toxicity. Based on these data, a decision was made to employ five (5) groups for the definitive study and to select the top dose, which would result in profound maternal toxicity, with three (3) additional dose levels of difethialone at which does were expected to survive.

4. Dose Administration

All doses were administered in a volume of 2 ml/kg of body weight/day prepared once during the dosing period. The dosing solutions were analyzed for concentration and stability. The following is from the executive summary of the analytical report (scanned from pages 49 and 50 of the investigators report):

A sample of difethialone (RTI Log No. 7660-08-01) was submitted for dosage formulation studies in support of RTI Project No. 65C-5724. The dosage formulation studies reported here included a mixing study of the test chemical and vehicle (corn oil) and a storage stability study (twenty-three days for the low dose and twenty-one days for the high dose).

Homogeneity studies conducted on a corn oil vehicle dosed with the test chemical at 0.3 and 15 µg/mL showed 3.5% variation in dose concentration among the three sampling locations for the low dose and 3.0% variation in dose concentration among the three sampling locations for the high dose. No problems were experienced with the preparation of neat chemical and vehicle dosage formulations. The stability studies conducted on solutions of the test chemical in corn oil at 0.3 and 15 µg/mL showed no loss of test chemical from the low dose formulation and a 3.8% loss of test chemical from the high dose formulation after storage at refrigerator temperature (3 to 9 °C) for 23 days and 21 days, respectively.

This report includes procedures for the preparation and storage of corn oil solutions of difethialone at concentrations of 0.3 to 15 µg/mL. The performance of the dosage analysis method was evaluated using standards in the range of 0.25 to 1.75 µg of test chemical/mL of corn oil. Linearity was confirmed (correlation coefficient = 0.9958). Accuracy was confirmed with relative errors of less than or equal to 9.6%; precision was demonstrated with relative standard deviations of less than or equal to 4.1% for triplicate determinations.

Dosage analyses were performed on dosage formulations mixed on November 30, 1993 and on March 28 and 30, 1994. The test chemical contents of the low doses were 89.3 and 102% of the nominal concentration, respectively. The test

chemical content for the November 30, 1993 low dosage formulation was originally reported to the study lab on December 2, 1993 as 90.9% of the nominal concentration. A mistake in data entry was subsequently discovered; correction of this error produced the value of 89.3%. No test chemical was detected in the samples of blank vehicle.

5. Observations

The following observations were conducted on the dams and fetuses (scanned from the investigators report page 13):

Clinical observations of all animals were made at least once daily on gd 0-6 (prior to dosing period) and on gd 20-30 (after dosing period) and twice daily, at dosing and at approximately one-two hours after dosing, throughout the dosing period (gd 7 through gd 19). Maternal feed consumption was evaluated from gd 3-7, 7-9, 9-12, 12-15, 15-19, 19-21, 21-24, 24-27, and 27-30.

On gd 30, approximately one to one and a half days before expected parturition, maternal animals were sacrificed by intravenous injection (marginal ear vein) of Sodium Pentobarbital Euthanasia-6 Injection CII (Anthony Products Co., Arcadia, CA), thoracic and abdominal cavities and organs examined, and their pregnancy status was confirmed by uterine examination. Uteri which presented no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites which may have undergone very early resorption (Salewski,1964). At sacrifice, the body, liver and uterus of each mated female were weighed, ovarian corpora lutea were counted, and uterine contents (*i.e.*, number of implantation sites, resorptions, dead fetuses, live fetuses) recorded. Live fetuses were dissected from the uterus and immediately euthanized by intraperitoneal injection of Sodium Pentobarbital Euthanasia-6 Injection Cii. All live fetuses were weighed and examined for external morphological malformations, including cleft palate, and variations. All live fetuses per litter were examined for visceral malformations and were sexed internally using a fresh tissue dissection method (Staples, 1974; Stuckhardt and Poppe, 1984). Approximately one-half of the live fetuses per litter were decapitated after dissection, and the heads were fixed and decalcified in Bouin's solution for free-hand sectioning and examination (Wilson, 1965). All fetal carcasses were eviscerated, macerated, stained with alcian blue/alizarin red S. and were examined for skeletal malformations and variations (Marr et al.,1988). After examination, all maternal and fetal organs and maternal carcasses were destroyed by incineration. Fetal carcasses were stored in glycerin:70% ethanol (1:1) following examination of skeletal structures; fetal head sections were stored in 70% ethanol solution.

Historical control data were provided to allow comparison with concurrent controls. From the investigators report (scanned from page 15):

A historical control data set for developmental toxicity studies with the New Zealand White rabbits in this laboratory during 1993-1994 is presented in Appendix III. This data set was developed at RTI when the decision was made to use rabbit does naturally mated by the supplier (previously this laboratory had artificially inseminated does on-site).

6. Statistical analysis

The following statistical analysis methods were employed (scanned from the investigators report, pages 13 and 14):

The unit of comparison was the pregnant female or the litter. Quantitative continuous data (*e.g.*, maternal body weights, fetal body weights, feed consumption, etc.) were compared among the four treatment groups and the one vehicle control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (*i.e.*, $p<0.001$), then nonparametric statistical tests were employed for the continuous variables (Winer,1962; see below). If Bartlett's test indicated homogeneous variances (*i.e.*, $p>0.001$), then parametric statistical tests were employed for the continuous variables. Parametric statistical procedures to be applied to selected measures from this developmental toxicity study were as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a, 1989b, 1990a, 1990b, 1990c) were used for the Analyses of Variance (ANOVA). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data, the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dosage-response relationship (Test for Linear Trend), and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ($p<0.05$) main effect for dosage occurred, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each difethialone-exposed group to the control group for that measure. A one-tailed test (*i.e.*, Dunnett's Test) was used for all pairwise comparisons to the vehicle control group except that a twotailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Nonparametric tests to be used on continuous data which did not have homogenous variances included the Kruskal-Wallis Test to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise comparisons to the designated control group, if the Kruskal-Wallis test was significant (Siegel, 1956). Jonckheere's test for k independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Nominal scale measures were analyzed by Chi-

Square Test for Independence for differences among treatment groups, and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p<0.05$) differences among groups, then a two-tailed Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each difethialone-dosed group and the control group (Snedecor and Cochran, 1967). A test for statistical outliers (SAS) was performed on maternal body weights and feed consumption (in g/day). If examination of pertinent study data did not provide a plausible biologically-sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data in g/day were negative for a given dam and period, they were designated "unrealistic" and excluded from summarization and analysis. If feed consumption data for a given observational interval (e.g., gd 7-9, 9-12, 12-15 or 15-19 during the treatment period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., treatment period, gd 7-19) also did not include this value.

C. Results

1. Maternal Toxicity

a. Mortality/Moribundity

Two females were nonpregnant (control and 10 µg/kg/day). Four females were removed due to dosing errors (control, 2.5, and 5.0 µg/kg/day) and one 2.5 µg/kg/day females was removed due to being without food. Five females aborted or had premature delivery (two control, one 2.5 µg/day and two 10.0 µg/kg/day). All animals in the 16 µg/kg/day dose group died or were sacrificed moribund (one at gd 14, 2 on gd 15, 2 on gd 16, 6 on gd 17, 4 on gd 18 and 1 on gd 19). In the 10 µg/kg/day dose group, 5 animals died or were sacrificed moribund (1 each on gd 18, 20, 21, 22, and 23.

b. Clinical Observations

Clinical observations were confined mainly to the 10 and 20 µg/kg/day dose groups and included...*external bleeding around mouth, ears, and the urogenital system, pale ears, nose, and lips/gums, and blood in pan beneath cage.* These signs were noted prior to death or sacrifice. No clinical signs considered to be related to treatment were noted in the 2.5 or 5.0 µg/kg/day dose groups.

c. Body Weight

The investigators supplied group mean data and individual animal data. The following table presents body weights and body weight gains data (from Table 2 , pages 23-26 of the investigators report):

Table I: Body Weights (grams)

Day	Dose: Control	2.5	5.0	10.0	20.0
0	3499±105	3604±82	3524±87	3504±88	3531±80
3	3453±84	3538±94	3422±73	3416±119	3472±80
7	3683±104	3771±103	3672±77	3708±122	3747±83
9	3617±94	3774±94	3635±84	3629±127	3715±85
12	3662±93	3823±89	3670±92	3658±116	3739±95
15	3676±101	3882±94	3752±94	3796±123	3845±104
19	3665±107	3904±95	3769±106	3758±136	
21	3682±116	3974±89	3882±103	3816±136	
24	3841±98	4044±85	3917±94	3768±139	
27	3922±95	4153±96	3945±98	3801±162	
30	3977±98	4213±97	4024±97	3852±167	
30 (Sac)	3947±97	4175±98	3993±97	3808±163	

Table II: Body Weight Gains (grams)

Group:	3-7	7-19	19-30	7-30	3-30	C3-30[1]
Control	230±48	-28±69	322±38†	448±65	524±58	-67.70±83.71
LDT	232±30	133±52	309±39	616±123	675±54	92.14±134.50
LMDT	250±31	98±43	254±30	469±44	602±55	-129.68±54.78
HMDT	292±28	97±46	147±44*	382±79	582±65	-175.30±72.24
HDT	not presented					

† = $p < 0.01$ for Linear Trend; * = $p < 0.05$; [1] = corrected body weight gains (body weight gain minus gravid uterus weight)

The high mid dose groups gained less weight during the post dosing period (gestation days 19 through 30, statistically significant and positive trend) and the low mid and high mid dose groups for the corrected body weight gains during the entire gestation period were depressed; however, effects were difficult to assess since the low dose gained much more weight than the control group lost.

d. Food consumption

The investigators supplied group mean data and individual animal data. The following table presents food consumption data (calculated by the review from data on Table 4, pages 32-34 of the investigators report):

Table III: Food Consumption Data (grams/animal/day)

Group:	3-7	7-19	19-30	3-30
Control	212.9±10.1	104.9±11.4	156.0±10.5†	144.4±9.3
LDT	224.8±9.3	136.3±12.0	174.0±9.4	164.8±8.9
LMDT	219.0±6.5	115.1±13.0	136.3±7.6	137.6±8.6
HMDT	222.6±11.2	119.5±12.0	131.3±15.4	129.6±11.6
HDT	224.8±7.1			

† = $p < 0.05$ for Linear Trend

The low mid and high mid dose groups consumed less food than the control during the post dosing period (gestation days 19-30) and for the overall gestation period. The following table presents food efficiency data in percent calculated by the reviewer from the above data:

Table IV: Food Efficiency Data (%)

Group:	3-7	7-19	19-30	3-30
Control	27.0	-2.2	18.8	13.4
LDT	25.8	8.1	16.1	15.2
LMDT	28.5	7.1	16.9	16.2
HMDT	32.8	6.8	10.2	16.6

Food efficiency was decreased in the high dose group during the post dosing period (gestation days 19-30). The dosing period effects were difficult to discern due to low food efficiency in the control group.

3. Gross Pathological Observations

The investigators supplied group summary and individual animal data. The following table presents the gravid uterine weights and absolute and relative maternal liver weights (from Table 2, pages 25-26 of the investigators report).

Table V: Organ Weights (grams)

Dose:Control	2.5	5.0	10.0
Gravid Uterine Weights			
516±37.87	524.16±47.04	598.68±28.03	557.30±35.22
Absolute Liver Weights			
116.53±5.22	133.76±8.33	115.92±	111.98±6.98
Relative (to body weight) Liver Weights (%)			
2.96±0.13	3.20±0.17	2.90±0.12	2.93±0.11

No treatment related effects were noted in the above data.

4. Cesarean section observations

The investigators supplied group summary and individual animal data. The following table presents the cesarean section observation data (from Tables 1 and 5, pages 23 and 35-37 and Appendices A-1 and A-4, pages 84-85 and 95-101 of the investigators report).

Table VI: Cesarean Section Observations

Dose:	Control	LDT	LMDT	HMDT	HDT
#Animals Assigned	16	16	16	16	16
#Animals Mated/Inseminated	16	16	16	16	16[1]
#Animals Pregnant	14	15	15	15	15[1]
Pregnancy Rate (%)	87.5	93.8	93.8	93.8	93.8[1]
Maternal Wastage					
#Died	0	0	0	4	15
#sacrificed(removed)	1	2	1	1	1
#Died/pregnant	0	0	1	0	15[1]
#Non pregnant	2	1	0	1	1[1]
#Aborted	2	1	0	2	0
# litters available	12	13	15	8	0
Total Corpora Lutea[1]	114	129	145	84	
Corpora Lutea/dam	9.50±0.77	9.92±0.73	9.67±0.54	10.50±0.57	
Total Implantations[1]	91	97	136	69	
Implantations/Dam	7.58±0.78	7.46±0.87	9.00±0.48	8.63±0.65	
Total Live Fetuses[1]	85	93	132	65	
Live Fetuses/Dam	7.08±0.65	7.15±0.92	8.73±0.55	8.13±0.48	
Total Resorptions	6	4	4	4	
Full	0	1	0	2	
Early	4	2	4	0	
Middle	1	0	0	0	
Late	0	0	0	2	
Total Dead Fetuses	1	1	0	0	
Resorptions/Dam(%)	5.41±2.65	3.98±2.23	3.98±1.94	4.69±3.29	
Mean Fetal Weight (g)	52.45±1.85	53.16±1.96	49.54±1.55	47.88±1.77	
Preimplantation Loss(%)[1]	20.2	24.8	6.2	17.9	
Postimplantation Loss(%)[1]	6.6	4.1	2.9	5.8	
Sex Ratio (%male)	50.64±5.39	40.02±4.72	52.21±4.11	46.79±6.87	

[1] = calculated by reviewer from individual animal data or group data.

No treatment related effects were noted in the above data (except for complete maternal deaths in the high dose group). The slightly reduced fetal body weights in the low mid and high mid dose groups were most likely due to the larger litter size as compared to the control group.

5. Developmental Toxicity

a. External Examinations

No treatment related effects were noted in the provided data. External malformations (designation by the investigators) included, one control fetus had meningocele and two other fetuses in one control litter had umbilical hernia. External variations (designation by the investigators) included, one control fetus with domed head.

b. Visceral Examinations

No treatment related effects were noted in the provided data. In the low dose the visceral malformations (designation by the investigators) included, one fetus had an aorta twice the normal size, another fetus had an interventricular septal defect, another fetus had hypoplasia of the semilunar valves and another fetus had pulmonary artery malformation with the artery not patent. Visceral variations (designation by the investigators) included one low mid dose fetus with bilateral partial enlarged lateral ventricle and two fetuses, one low mid dose (a different litter) and a high mid dose, with an abnormal number of papillary muscles.

c. Skeletal Examinations

Table V: Skeletal Examinations[a]

Observations+	Control	LDT	LMDT	HMDT
#pups/litters examined	84/12[b]	93/13	131/15	65/8
#pups/litters affected				
variations[1]	51/12	58/12	74/14	45/8
malformations[1]	3/1	2/1	0/0	0/0
Fused sternebrae	1/1	2/1	0/0	0/0
Branched rib	1/1	0/0	0/0	0/0
Fused ribs	1/1	0/0	0/0	0/0
Centrum 1/2 normal size: Thoracic	1/1	0/0	0/0	0/0
Misaligned centrum: Thoracic	1/1	0/0	0/0	0/0
Frontals and parietals unossified at the midline	1/1	0/0	0/0	0/0
Rib on Lumbar I:				
Bilateral full	31/12	30/10	49/14	28/8
Left full	8/7	13/10	9/5	6/4
Right full	4/4	2/2	3/2	3/2
Bilateral rudimentary	3/2	8/4	6/3	5/3
Left rudimentary	4/4	5/5	7/5	3/3
Right rudimentary	6/4	11/9	5/3	6/4
Normal cartilage, bipartite ossification center:				
Thoracic centrum	0/0	1/1	0/0	0/0
Normal cartilage, unilateral ossification center:				
Thoracic centrum	1/1	0/0	0/0	0/0

[1] = designation by the investigators; + = some observations may be grouped together; b = fetal/litter incidence

[a] = Data extracted from Study No. 65C-5724-05/06, Table 7, pages 42-44.

No treatment related effects were noted.

D. Discussion/Conclusions

a. Maternal Toxicity:

Maternal toxicity was noted in the high mid and high dose groups as increased mortality with associated clinical observations related to hemorrhage prior to death.

b. Developmental Toxicity:

Since all dams died in the high dose, possible developmental toxicity could not be determined for that dose; therefore, this will be considered as the developmental toxicity LOEL.

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

No treatment related effects were noted.

iv. Malformations:

No treatment related effects were noted.

E. Study Deficiencies:

No major study deficiencies were noted.

F. Core Classification: Acceptable.

Maternal Toxicity NOEL = 5.0 µg/kg/day
Maternal Toxicity LOEL = 10.0 µg/kg/day
Developmental Toxicity NOEL = 10.0 µg/kg/day
Developmental Toxicity LOEL = 20.0 µg/kg/day

OBJECTIVES

The present study was designed to evaluate the potential of difethialone to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in New Zealand White rabbits.

MATERIALS AND METHODS

Test Chemical, Dosage Formulations and Analyses

The test chemical, difethialone technical [(Bromo-4'-[Biphenyl-1-1']-yl-4) 3-Tetrahydro-1,2,3,4-Naphthyl-1]3-Hydroxy-4,2H-1-Benzothiopyran-one-2; CAS No. 104653-34-1) was received at Research Triangle Institute (RTI) Materials Handling Facility from LiphaTech, Inc., Milwaukee, WI, in three shipments. For all shipments as provided by the supplier, the Lot No. was DIF8011 and the Analysis No. was 28412M; the appearance was a white-pinkish odorless powder with a purity of 100.95%, as provided by the supplier (see Certificate of Analysis, Appendix I). The first shipment was received at RTI on September 13, 1993 in five (5) amber vials wrapped in aluminum foil with screw caps (10 mg/vial); the second shipment was received at RTI on October 21, 1993, in three (3) amber vials wrapped in aluminum foil with screw caps (10 mg/vial); the third shipment was received at RTI on March 17, 1994 in one 500 mg amber glass vial wrapped in aluminum foil with a snap-on cap. All shipments (all vials) received the RTI Log Book No. 7660-08-01. Difethialone was stored at room temperature under controlled conditions, protected from light and moisture.

A range-finding study was performed to establish the dose-response range of difethialone in New Zealand White rabbits for use in the developmental toxicity study. A detailed report of this rangefinding study can be found in Appendix V. The study design involved five (5) treatment groups and a vehicle control group, each comprised of five (5) rabbits at doses of 0.0, 1.25, 2.5, 5.0, 15.0 and 30.0 µg/kg/day. In addition, there were four (4) treatment groups and a vehicle control group, each comprised of three (3) naturally bred rabbits designated as satellite females, at doses of 0.0, 0.625, 1.25, 2.5 and 5.0 µg/kg/day. The dosing period was gd 7 through 19. Satellite females were handled, treated and examined exactly like study females until gd 20 at which time satellite females were euthanized for Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) analyses. On gd 30, all surviving study females were euthanized, weighed and necropsied. All live fetuses were weighed, examined for external malformations (including cleft palate) and variations, and discarded.

Maternal mortality and morbidity were observed at 15.0 and 30.0 µg/kg/day. There were no treatment- or dose-related clinical observations at doses of 0.0 through 5.0 µg/kg/day. The clinical signs seen at 15.0 and 30.0 µg/kg/day were internal bleeding externalized from the nose and/or mouth and

vagina, external bleeding at the site of the eartag, pallor and ultimately morbidity and death. There were no changes in PT or APTT in any dose examined. At 15.0 µg/kg/day, there were significant increases in resorptions per litter, in nonlive (resorptions plus dead) per litter and in adversely affected (nonlive plus malformed) per litter, all due to the increased resorptions. The percentage of litters with one or more resorptions exhibited a dose related upward trend and the percentage of litters with nonlive and adversely affected implants was significantly increased at 15.0 µg/kg/day. There were no treatment- or dose-related changes in pre- or post-implantation loss, incidence of nonlive or adversely affected implants per litter, of the number of live fetuses per litter, of fetal body weight per litter in any of the dose groups, 0.0 through 5.0 µg/kg/day. Litter size and mean fetal body weight per litter were reduced at 15.0 µg/kg/day. External examination of all live fetuses indicated no malformations or variations detected in this study.

In this preliminary study, doses of 30.0 or 15.0 µg/kg/day were toxic to the does, resulting in high levels of mortality. In contrast, doses of 1.25 through 5.0 µg/kg/day resulted in no detectable maternal or developmental toxicity. Based on these data, a decision was made to employ five (5) groups for the definitive study and to select the top dose, which would result in profound maternal toxicity, with three (3) additional dose levels of difethialone at which does were expected to survive.

The nominal doses employed for this study were 0.0, 2.5, 5.0, 10.0, and 20.0 µg/kg/day, equivalent to 0.0, 1.25, 2.5, 5.0 and 10.0 µg/ml at a dosing volume of 2.0 ml/kg. Prior to study dose formulation, formulations encompassing the range of doses employed in the study (0.3 and 15.0 µg/ml) were assayed for homogeneity, stability and dose level verification. Dosing formulations were homogeneous and stable for at least 21 days under refrigerated conditions; solutions were formulated once during the study and used within the established stability time limit. To prepare dosing formulations, difethialone was dissolved in corn oil (Mazola®); CAS No. 8001-30-7); the concentration was determined by the following formula:

$$\text{Concentration } (\mu g/ml) = \frac{\text{Dose level } (\mu g/kg)}{\text{Dose volume } (2 \text{ ml/kg})}$$

Difethialone was soluble in corn oil at all dose levels tested. The concentration of difethialone in the dosing solutions varied with dose with the dosing volume held constant. The top dose solution, 10.0 µg/ml (equivalent to 20.0 µg/kg/day at a dosing volume of 2 ml/kg), was formulated in amber bottles in corn oil with sonication and stirring. Solutions of difethialone in vehicle were formulated by serial dilution with corn oil from the top dose formulation and were prepared once in a quantity sufficient for use for the entire dosing period. Bulk amber bottles of dose formulations were stirred with caps on each day prior to dosing. Aliquots of the lowest formulation of difethialone and vehicle were analyzed to verify the concentration of the test compound prior to use of the formulation. Dosing formulations with assay

values of 90-110% of nominal were considered to be suitable for use in these studies. Personnel, other than the laboratory supervisor and those involved in the formulation and analyses of dosage formulations for difethialone concentration, were not informed of the formulation concentrations until all laboratory work had been completed.

For analysis of the dosing formulations, three replicate 1.0 ml samples of the vehicle control solution (corn oil) and of the lowest dosing formulation, 1.25 µg difethialone/ml, were transferred into silanized 1-dram amber vials and diluted with 1.0 ml hexane. Each sample was applied to a 3 cc Florisil Solid Phase extraction column (Varian Assoc.), pre-rinsed with 5.0 ml hexane. After multiple rinsing with hexane and methylene chloride/hexane (25:75), the eluate was discarded. The test material was then eluted from each column with 8 ml methylene chloride into separate silanized 2 dram vials. A total of 50 pl of internal standard solution (0.600 mg 1-chloroanthraquinone/ml of methylene chloride/methyl-t-butyl ether [1:1]) was added to each eluate and the samples were evaporated to dryness. Each residue was then reconstituted in 0.3 ml hexane, sonicated and transferred to silanized limited volume inserts for analysis (see below). Standard solutions were prepared by weighing 3.50 mg (Solution A) and 2.50 mg (Solution B) difethialone into 100 ml methylene chloride/hexane (1:1) with sonication and stirring for stock solutions; multiple dilutions were made with methylene chloride/hexane (1:1) to obtain standard solutions of 2.50-17.5 µg difethialone/ml. Aliquots (100 µl) of the dilutions were evaporated to dryness, and dissolved in 1.0 ml corn oil to produce final standards of 0.250-1.75 µg difethialone/ml of corn oil. The standard solutions were then extracted as described above.

The extracted standard and dosing solution samples were analyzed by high performance liquid chromatography (Waters 845 HPLC) using a Normal Phase, Supelcosil LC-Si:(25 cm x 4.6 mm I.D.) column; the detector was UV (Waters 490) at 325 nm.

Details of analytical procedures and results are found in Appendix I.

Animals and Husbandry

The test animals were New Zealand White (NZW) rabbits supplied by Hazleton Research Products, Inc. (Denver, PA). Eighty-one (81) female rabbits naturally mated by the supplier (date of mating designated gestational day (gd) 0; Hafez, 1970) were purchased for this developmental toxicity study. Female rabbits were approximately five months of age (date of birth, October 23, 1993), approximately 2792 - 4158 g in weight on gd 0 as provided by the supplier and at gd 1 (40 females) and gd 2 (41 females) upon arrival at RTI. Eighty (80) mated female rabbits were used in this study (i.e., 5 groups of 16 mated does each). One female rabbit not utilized (extra animal) was euthanized by intravenous injection (marginal ear vein) of Sodium Pentobarbital Euthanasia-6 CII Injection (Anthony Products, Inc., Arcadia, CA) and discarded.

Animals were randomly assigned to cages upon receipt and health status was determined by a veterinarian within two days of receipt. Animals were housed in two animal rooms (see below), distributed between the two rooms by gestational date and dose groups (representative of each group and date in each room). Quarantine for vendor-mated rabbits from this supplier was limited to the five-six days prior to dosing with the approval of the Animal Research Facility Veterinarian. Females were housed singly in stainless steel cages with mesh flooring (Hoeltge, Inc., Cincinnati, OH) (19 x 24 x 18 inches or 24 x 24 x 20 inches). Feed (#5322 Purina Certified Rabbit Chow®) was rationed at 65 g the first 24 hours for all females, at 125 g for those females at gd 2 and ad libitum for all females from gd 3 to scheduled sacrifice. Deionized/ filtered tap water, via plastic or glass water bottles, butyl rubber stoppers and stainless steel sipper tubes, from the Durham, North Carolina water system was available ad libitum throughout the study. The analysis of the rabbit chow for chemical composition and possible chemical contamination and analysis of the drinking water was provided by the suppliers. Contaminant levels were below certified levels for both feed and water and they did not affect the design, conduct or conclusions of this study. Rabbit chow was stored at approximately 60-70°F and the period of use did not exceed six months from the milling date. At all times, animals were housed, handled and used according to the NIH Guide for the Care and Use of Laboratory Animals (NIH,1985).

Environmental conditions were continuously recorded and controlled using an automatic system (Barber-Coleman Network Supervisor System, Diversified Environmental Control, Inc., Greensboro, NC) during the course of the study. The animal rooms used for this study, Animal Research Facility Rooms 207 and 209, were maintained on a 12:12 hour light:dark cycle. Target conditions for temperature and humidity in the animal room were 61-69°F and 40-60%, respectively. Animal room 207 was maintained at a mean temperature of 65.2 + 0.8 (SD)°F with a range of 63.5-68.8°F except for two hours up to 73.0°F on April 2,1994, two hours up to 69.2 on April 20,1994, and for 15 consecutive hours on April 25-26,1994 up to 81.7°F. The mean relative humidity for room 207 was maintained at 51.3 + 2.3 (SD)% with a range of 44.3 - 59.2% except for one hour at 61.8% on April 6,1994, one hour at 60.3% on April 21,1994 and one hour at 60.6% on April 25,1994. Animal room 209 was maintained at a mean temperature of 66.0 + 1.8 (SD)°F with a range of 63.9-68.7°F except for three hours up to 74.2°F on April 2,1994, one hour at 69.5°F on April 6,1994 and two hours up to 73.2°F on April 20,1994. The mean relative humidity for room 209 was maintained at 55.3 + 2.8 (SD)% with a range of 46.0 - 59.9% except for one hour at 60.9% on March 30,1994, two discontinuous hours at 63.3% and 60.0% on April 2,1994, one hour at 62.2% on April 7,1994, one hour at 63.5% on April 8,1994, one hour at 60.1% on April 14,1994, one hour at 61.1% on April 18,1994, two hours up to 63.5% on April 19,1994, one hour at 61.8% on April 21,1994, one hour at 63.2% on April 22,1994, four hours up to 60.6% on April 24,1994, two hours up to 67.9% on April 26, 1994, and one hour at 60.5% on April 27, 1994. For room 209 from 1:30 p.m. on April 2, to 9:36 a.m. on April 3, 1994, there were no data collected due to problems in the recording computer which constitutes a protocol deviation; any excursions were monitored

and alarmed during this time. The relatively brief and minor excursions outside the protocol-mandated ranges for temperature and relative humidity did not affect the design, conduct or conclusions of this study.

All maternal rabbits were individually identified by ear tag by the supplier prior to arrival at RTI. In addition, each mated female received a dam study number. All data generated during the course of this study were tracked by these numbers.

Mating

Rabbits to be placed on study were naturally mated by the supplier prior to shipment. Forty-one (41) and 40 females were mated on each of two days, and arrived at RTI on gd 1 (40 females) and gd 2 (41 females). The female eartag, gd 0 date and body weight, and the male ID for each mated female were supplied by the vendor.

Study Design and Treatment

The study was conducted with four (4) treatment groups and a corn oil vehicle control group, each comprised of 16 mated rabbits. The dates of performance were as follows: the females were naturally mated (day designated gd 0) by the supplier on March 27 (41 females) and March 28, 1994 (40 females). The mated females arrived at RTI on March 29, 1994 (41 females on gd 2, 40 females on gd 1); dosing dates (gd 7 through 1 9) were April 3-16, 1994. Scheduled sacrifice dates (gd 30) were April 26 and 27, 1994.

The doses were 0.0, 2.5, 5.0, 1 0.0 and 20.0 µg/kg/day based on a range-finding study in pregnant rabbits performed at RTI (Appendix V). The highest dose level was chosen to induce overt maternal toxicity. The low dose was selected to be a maternal and developmental No Observable Adverse Effect Level (NOAEL). The mid doses were between the high and low doses, to allow evaluation of dose-response relationships, were such to occur. See Text Table A for summarization of study design and doses.

Text Table A. Study Design and Doses

Group Number	5 Digit Rx Code	Color Code	No. Mated Females	Dosing Period (13 consecutive days)	Nominal Concentrations (µg/kg/day)	(ug/ml)	Dosing Volume (ml/kg)
1	61642	purple	16	gd 7-19	0.0	0.0	2.0
2	15059	orange	16	gd 7-19	2.5	1.25	2.0
3	32388	blue[a]	16	gd 7-19	5.0	2.5	2.0
4	88072	yellow	16	gd 7-19	10.0	5.0	2.0
5	25940	red	16	gd 7-19	20.0	10.0	2.0

[a] All study records have a blue color code; inadvertently, chemistry records have a black color code (all documented in the study records).

Naturally mated rabbits (does) were assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights across dose groups on gd 3 (rabbits arrived at RTI on gd 1 and 2). On gd 0, maternal body weights (provided by the supplier) ranged from 2792 to 4158). Difethialone in corn oil or corn oil alone was administered by gavage to mated females from gd 7 through gd 19, at a volume of 2 ml/kg, based on each animal's most recent body weight. Maternal animals were weighed on gd 0 (provided by the supplier), 3, 7, 9, 12, 15, 19, 21, 24, 27 and 30. The dosing solutions were administered via a 10.0 cc syringe attached to a 13 gauge, 6 inch curved dosing needle (Perfektum®, Popper and Sons, New Hyde Park, NY).

Clinical observations of all animals were made at least once daily on gd 0-6 (prior to dosing period) and on gd 20-30 (after dosing period) and twice daily, at dosing and at approximately one-two hours after dosing, throughout the dosing period (gd 7 through gd 19). Maternal feed consumption was evaluated from gd 3-7, 7-9, 9-12, 12-15, 15-19, 19-21, 21-24, 24-27, and 27-30.

On gd 30, approximately one to one and a half days before expected parturition, maternal animals were sacrificed by intravenous injection (marginal ear vein) of Sodium Pentobarbital Euthanasia-6 Injection CII (Anthony Products Co., Arcadia, CA), thoracic and abdominal cavities and organs examined, and their pregnancy status was confirmed by uterine examination. Uteri which presented no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites which may have undergone very early resorption (Salewski,1964). At sacrifice, the body, liver and uterus of each mated female were weighed, ovarian corpora lutea were counted, and uterine contents (*i.e.*, number of implantation sites, resorptions, dead fetuses, live fetuses) recorded. Live fetuses were dissected from the uterus and immediately euthanized by intraperitoneal injection of Sodium Pentobarbital Euthanasia-6 Injection CII. All live fetuses were weighed and examined for external morphological malformations, including cleft palate, and variations. All live fetuses per litter were examined for visceral malformations and were sexed internally using a fresh tissue dissection method (Staples, 1974; Stuckhardt and Poppe, 1984). Approximately one-half of the live fetuses per litter were decapitated after dissection, and the heads were fixed and decalcified in Bouin's solution for free-hand sectioning and examination (Wilson, 1965). All fetal carcasses were eviscerated, macerated, stained with alcian blue/alizarin red S. and were examined for skeletal malformations and variations (Marr et al.,1988). After examination, all maternal and fetal organs and maternal carcasses were destroyed by incineration. Fetal carcasses were stored in glycerin:70% ethanol (1:1) following examination of skeletal structures; fetal head sections were stored in 70% ethanol solution.

Statistics

The unit of comparison was the pregnant female or the litter.

Quantitative continuous data (*e.g.*, maternal body weights, fetal body weights, feed consumption, etc.) were compared among the four treatment groups and the one vehicle control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (*i.e.*, $p<0.001$), then nonparametric statistical tests were employed for the continuous variables (Winer,1962; see below). If Bartlett's test indicated homogeneous variances (*i.e.*, $p>0.001$), then parametric statistical tests were employed for the continuous variables. Parametric statistical procedures to be applied to selected measures from this developmental toxicity study were as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a, 1989b, 1990a, 1990b, 1990c) were used for the Analyses of Variance (ANOVA). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data, the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dosage-response relationship (Test for Linear Trend), and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ($p<0.05$) main effect for dosage occurred, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each difethialone-exposed group to the control group for that measure. A one-tailed test (*i.e.*, Dunnett's Test) was used for all pairwise comparisons to the vehicle control group except that a twotailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Nonparametric tests to be used on continuous data which did not have homogenous variances included the Kruskal-Wallis Test to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise comparisons to the designated control group, if the Kruskal-Wallis test was significant (Siegel, 1956). Jonckheere's test for k independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Nominal scale measures were analyzed by Chi-Square Test for Independence for differences among treatment groups, and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p<0.05$) differences among groups, then a two-tailed Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each difethialone-dosed group and the control group (Snedecor and Cochran, 1967). A test for statistical outliers (SAS) was performed on maternal body weights and feed consumption (in g/day). If examination of pertinent study data did not provide a plausible biologically-sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data in g/day were negative for a given dam and period, they were designated "unrealistic" and excluded from summarization and analysis. If feed consumption data for a given observational interval (e.g., gd 7-9, 9-12, 12-15 or 15-19 during the treatment period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., treatment period, gd 7-19) also did not include this value.

Personnel

The evaluation of difethialone for developmental toxicity in New Zealand White rabbits was conducted at Research Triangle Institute (RTI), Research Triangle Park, NC, under contract to LiphaTech, Inc., Milwaukee, WI; Mr. E. F. Marshall, Director of Technical Services, was the Sponsor's Representative. Dr. R. L. Baron, Baron Associates, Raleigh, NC, was the Study Monitor. The RTI personnel indicated below contributed to the completion of this study.

Dr. R. W. Tyl served as Study Director. Developmental toxicology personnel included Ms. M. C. Marr (Laboratory Supervisor), Ms. C. B. Myers (Data Specialist), Ms. F. S. Gerling (Study Team), Ms. V. I. Wilson (Study Team), Ms. D. L. Holtgrefe, Ms. K. E. Prince, Ms. L. B. Pelletier, Ms. E. M. Guill, Ms. M-S. Perry, and Ms. N. M. Kuney. Bulk chemical handling and dosage formulations were provided by Mr. M. M. Veselica (Supervisor, MHF), Mr. D. L. Hubbard, Mr. R. T. Price, and Mr. T. D. Burnette. Analyses of dosing formulations were done by Ms. D. R. Brine, Ms. M. E. Parker and Mr. D. R. Briggs. Ms. A. F. Gilliam performed the measures of coagulation function in the range-finding study (Appendix V). Animal care was provided by Dr. D. B. Feldman, ACLAM, Animal Research Facility (ARF) Veterinarian, and Mr. F. N. Ali, MBA, LATG, ARF Supervisor. Quality Assurance personnel were Ms. S. M. Taulbee (Manager), Ms. C. D. Keller, Ms. P. D. Hall, Mr. S. Sherrill and Mr. D. L. Brodish.

The final report was prepared by Dr. R. W. Tyl with assistance from Ms. C. B. Myers, Ms. M. C. Marr and Ms. F. S. Gerling on data compilation and statistical analyses. The individual scientist reports were prepared and signed by the author(s).

The protocol and two amendments detailing the design and conduct of the study are presented in Appendix IV. The protocol was signed by the Study Director on September 15, 1993.

Historical Control Dataset

A historical control data set for developmental toxicity studies with the New Zealand White rabbits in this laboratory during 1993-1994 is presented in Appendix III. This data set was developed at RTI when the decision was made to use rabbit does naturally mated by the supplier (previously this laboratory had artificially inseminated does on-site).

Storage of Records

All original data sheets for the present study are stored in the RTI Archives, under the control of the RTI Quality Assurance Officer, along with all biological samples collected during the course of the study which remain the responsibility of RTI. Work sheets and computer printouts which were generated in the statistical analysis of data are stored in the RTI Archives.

Copies of this report are filed with the RTI Archives as well as with the Sponsor, LiphaTech, Inc., Milwaukee, Wi.

Compliance

The study was performed in compliance with the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) testing guidelines (U.S. EPA, 1988) and reported in compliance with the Standard Evaluation Procedures for Developmental Toxicity Studies (U.S. EPA, 1993).

All records, data and reports will be maintained in storage for the registration lifetime of the chemical or for as long as the quality of the preparation affords evaluation, whichever is less.

The toxicology laboratories at RTI are operated in compliance with FIFRA Good Laboratory Practice Standards (U.S. EPA, 1989). The RTI Animal Research Facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). This study was conducted in compliance with the FIFRA GLP regulations and AAALAC accreditation standards. The Toxicology Laboratories at RTI are also approved by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan, for toxicology studies on agricultural chemicals. The approval letter from MAFF, No. 623, was dated May 19, 1992 (see Appendix I for copy of approval letter).

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